APPLICATION OF EXPERIMENTAL DESIGN FOR PHOTODEGRADATION OF ROSE BENGAL (ACID RED 94)

MUHAMAD ZULHELMI BIN MOHAMAD FITHOL

A thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

April 2009

I declare that this thesis entitled "Application of Experimental Design for Photodegradation of Rose Bengal (Acid Red 94)" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature	:………………………………
Name	: Muhamad Zulhelmi Bin Mohamad Fithol
Date	: 30 April 2009

Special Dedication to my beloved mother; Aminah Binti Din
and my hardworking father; Mohamad Fithol Bin Abdullah,

For all your endless care, support and trust in me.

ACKNOWLEDGEMENT

First and foremost, I would like to thank God for bestowing His grace and bringing wisdom on me until I finish my reseach. Next, I wish to express my biggest gratitude towards my thesis supervisor, Mr. Rozaimi Bin Abu Samah for his valuable guidance and support throughout my research. My appreciation also goes to my fellow friends for your cooperation and encouragement.

I am very thankful to Universiti Malaysia Pahang (UMP) for providing good facilities regarding my research in the campus. To all the technical unit staff in Faculty of Chemical & Natural Resources Engineering, thank you for your instructions and guidance.

My sincere appreciation also extends to all my fellow colleagues and others who have provided assistance at various occasions. Thank you for the time sacrificed to accompany me. And last but not least, I am grateful to all my family members. Without their constant encouragement and support, I would not have the concentration on doing a good job.

I hope this research will give the readers some insight as to the application of experimental design as well as dyes decolorization studies.

ABSTRACT

Advance Oxidation Process (AOP) of Acid Red 94 (AR 94) by UV and H_2O_2 system were carried out in this study. AR 94 was irradiated with UV light in the presence of H_2O_2. The photodegradation process of the dye was monitored spectrophotometrically. Effects of AR 94 and H_2O_2 concentrations, pH, and irradiation time for photodegradation of AR 94 were investigated throughout this research while other experimental conditions were fixed at specific values. Statistical approach was employed to study the effect of selected parameters with the aid of Design-Expert® 7.1.6 software. Two level factorial design was employed for the experimental design. From the result, it is shown that the highest percent of AR 94 degradation can be achieved was 92.31%. From the analysis of variance, it is found that the AR 94 concentration, pH, and time were significant factors along with the interaction factors of AR 94 concentration, H_2O_2 concentration, and pH which gave significant effect for AR 94 degradation as well. Then, the optimization process was done using response surface methodology (RSM). Based on ANOVA result, the proposed model can be used to navigate the design space. It was found that the response of AR 94 decolorization is very sensitive to the independent factor of pH. The proposed model for central composite design fitted very well with the experimental data with R^2 and R^2_{adj} correlation coefficients of 0.976 and 0.943, respectively. Analysis of results data shown that the optimum conditions suggested by the design of experiment were; 20μM AR 94, 0.05 M H_2O_2, 3.75 pH value and irradiation time 30 minutes.

ABSTRAK

Proses pengoksidaan *Acid Red 94* (AR 94) oleh sistem UV dan H_2O_2 telah dijalankan di dalam kajian ini. Radiasi cahaya UV dengan kehadiran H_2O_2 telah dikenakan ke atas AR 94. Proses fotodegradasi bagi AR 94 dipantau secara spektrofotometrik. Kesan-kesan kepekatan AR 94 dan H_2O_2, pH, dan masa radiasi untuk fotodegradasi AR 94 telah diselidik sepanjang penyelidikan ini manakala faktor-faktor eksperimen lain telah ditetapkan pada nilai-nilai yang khusus. Pendekatan statistik telah digunakan untuk mengkaji kesan terhadap kesan-kesan yang ingin dikaji dengan bantuan perisian Design-Expert®7.1.6. Rekabentuk faktoran dua-faktor telah dipilih dalam merekabentuk eksperimen. Hasil eksperimen menunjukkan, peratus degradasi AR 94 tertinggi yang boleh dicapai ialah 92.31%. Daripada analisa varians, didapati kepekatan AR 94, pH, dan masa radiasi adalah faktor yang penting di samping interaksi faktor antara kepekatan AR 94, kepekatan H_2O_2, dan nilai pH juga memberi kesan terhadap degradasi AR 94. Kemudian, proses pengoptimuman dibuat menggunakan Metodologi Permukaan Sambutan. Berdasarkan hasil dari ANOVA, model yang dicadangkan boleh digunakan untuk memanipulasi rekabentuk ruang. Hasil eksperimen mendapati bahawa respon fotodegradasi AR 94 sangat sensitif terhadap faktor tak bersandar pH. Model yang dicadangkan dengan Rekabentuk Gubahan Memusat juga sesuai dan selari dengan data eksperimen dengan nilai korelasi koeffisien R^2 and R^2_{tlrs} masing-masing 0.976 dan 0.943. Analisis bagi data hasil eksperimen menunjukkan keadaan optimum yang dicadangkan oleh aplikasi rekabentuk eksperimen adalah; 20μM AR 94, 0.05 M H_2O_2, nilai pH 3.75, dan 30 minit masa radiasi.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS/ABBREVIATIONS

ANOVA	-	Analysis of variance
AR 94	-	Acid Red 94
H_2O_2	-	Hydrogen Peroxide
min	-	minutes
UV	-	Ultraviolet
R^2	-	Regression correlation
μM	-	micromolar
M	-	molar
AOP	-	Advanced Oxidation Process
%	-	percentage
°C	-	degree Celsius
RSM	-	response surface methodology
C. I number	-	colour index number
HCl	-	hydrochloric acid
NaOH	-	sodium hydroxide
Sqrt	-	square root
nm	-	nanometer
R^2	-	regression correlation/korelasi koeffisien
R^2_{adj}.	-	adjusted regression correlation
R^2_{tlrs}	-	korelasi koeffisien terlaras

CHAPTER 1

INTRODUCTION

1.1 Background

Nowadays, synthetic dyes have been widely used in many industrial processes especially in the textile industry, paper and printing, and plastics industry (Körbahti and Rauf, 2008a). Synthetic dyes are classified according to their predominant chemical structures. The structural varieties of dyes include; acidic, reactive, basic, disperse, azo, diazo, anthraquinone-based, and metal complex. These dyes have a very complex structures and low biodegradability (Bali, 2004). In addition, the highly structured polymers of these dyestuffs cause huge threat to the environment. During the production process, there was estimated around 1 – 15 % of the dyes found in the effluent (Körbahti and Rauf, 2008a). Due to its complex structures and low biodegradability, most of the dyes present in the effluent also could be carcinogenic due to their precursors and degradation products; for example azo dyes which have big percentage of synthetic dyes and degraded into carcinogenic amines (Bali *et al*., 2004).

Numerous efforts and research have been made to remove these dangerous chemical compounds. There are many traditional techniques applied in the removal process such as coagulation and flocculation, activated carbon adsorption, membrane filtration, and sedimentation. However, these methods just convert the wastewater containing dyes into secondary waste in solid form. This secondary waste has to be either treated again or dumped as such. Recent studies shown that advanced oxidation processes (AOPs), like UV/H_2O_2 (Behnajady *et al*., 2006), photocatalytic (Zhao *et al.*, 1998), Fenton and photo-Fenton processes (Çatalkaya and Şengül, 2006) and (Bali *et al*., 2004), result in promising solution towards dyestuffs detoxification and color removal. AOP based on the H_2O_2/UV system has produced high efficiency in the degradation of several types of dye that present in the industrial effluent. UV/H_2O_2 system produces ●OH radicals that become strong oxidizing agent to degrade dyes polymer into unharmed and safe substance to be discharge into the environment (Abdullah *et al*., 2007), (Bali, 2004), (Bali *et al*., 2004), and (Shu *et al.*, 2004).

The classical and conventional methods of studying the process by maintaining the other factors at unspecified constant level cannot measure the combination of parameters that affecting the experiment results. These methods also consume more time and required numerous amounts of experiments to represent the combinational effect of the parameters. With the large number of experiment, the result will be unreliable. These limitations of conventional methods can be solved by optimizing the important parameters using response surface methodology (RSM). RSM is a collection of mathematical and statistical techniques for developing, improving and optimizing processes and can be used to screen the important parameters and compute the combinational effect even with the complex interaction between parameters. RSM is to determine the optimum condition for specified parameters and to predict the future response using the response surface model. The application of statistical experimental design techniques can improved product yields, reduced process variability and experimental time; cost effective. The design also troubleshoots process problems and makes the process "robust" against external and non-controllable factors. "Robust" means relatively insensitive to these factors or influences (Montgomery, 1997). Thus the interaction between the parameters is studied and optimized using the response surface methodology.

1.2 Problem Statement

Untreated dye effluent produced by industrial process is highly colored and possesses dangerous characteristics such as high toxicity, carcinogenic in nature, low biodegradability, and reduce sunlight penetration. It also inhibits aquatic microorganism growth and threatening the flora and fauna stability. Moreover it can cause intestinal cancer and cerebral abnormalities in fetuses for mammals especially human.

Due to the high level risk by untreated dye effluent, many treatment methods and strategies have been used to degrade the dye and minimized the risk. The application of conventional methods; coagulation/flocculation, filtration, activated carbon adsorption, sedimentation, etc. does not totally degrade the dye effluent. The biological treatment is not a solution to this problematic due to the low biodegradability or toxicity of some dyes. Meanwhile, the chemical methods have not produce sufficient reduction in organic matter and not adequate enough for decoloration of dyes. Furthermore, both methods produced secondary pollutants that required further treatments.

The photocatalytic reaction is favor over other conventional and classical method for degradation of dyes because its simplicity of the system and full degradation of dyes based on the generation of highly reactive hydroxyl radicals, appear as emerging alternatives for the mineralization of organic pollutants. The system also promises a good efficiency for the degradation process. In addition, the system does not required further treatment although using chemical such as H_2O_2. This will save the operation cost for promising wastewater treatment.

Literally, Rose Bengal (C.I. name is Acid Red 94) dye has been used in numerous application in various areas such as laboratory research, biomedical, and biological application. Despite the numerous applications, information on its photolytic decolorization is not yet available in literature. Thus, it is very important to initiate the study on Acid Red 94 (AR94) since it possessed huge threat to the environment same as the other industrial dyes application.

In this study, the UV/H_2O_2 system will be used to degrade AR 94 while the optimum condition for the photodegradation of AR 94 will be determine using Response Surface Methodology (RSM) method with the aid of Design Expert® 7.1.6 software.

1.3 Objective

The objective of this research is to improve the Acid Red 94 decolorization using experimental design application.

1.4 Scopes of Study

This study will cover following scopes:

i. Using of full factorial design to screen the significance parameters for the Acid Red 94 decolorization.
ii. Application of Response Surface Methodology for optimizing the process of photodegradation of Acid Red 94.

CHAPTER 2

LITERATURE REVIEW

2.1 Dyes and Pigments

2.1.1 History

Dyes for thousand of years literally originated from vegetable or animal resources. Roots, berries, flower, insects, and crustacean cells were combined with minerals called mordants to get the desired colours that ranged based on the colour spectrum. No organic source was considered too off-beat if it produced a quality and satisfactory colours. A change in colorant history occurred in 1856, when English chemist William Henry Perkin (1838–1907) discovered a way to produce a dye in the laboratory when he tries to synthesize quinine (the only effective antimalaria treatment present) using coal tar. Although he was not successful, but he focuses his studies on the reaction of other coal tar bases including a mixture of aniline and toluidine. After that, he was able to produce the crude bases in the presence of MeOH. That dye, mauve, was produced from materials found in common coal tar. Perkin's discovery showed chemists that dyes and pigments could be produced synthetically (Zollinger, 2004). Periodically, a numerous and wide variety of colours have flooded into the world of dye and textile.

2.1.2 Dyes

Dyes are an important class of chemicals which are widely used in many industrial processes such as the leather, textile, and printing industries (Rauf *et al*., 2007). There are various kinds of dyes available in market such as azo, anthraquinone, triarylmethane, diarylmethane, acridine, quinine, xanthenes, and nitro dyes. These dyes are extensively used to impart colour to various industrial applications (Rauf *et al*., 2008). Shigwedha *et al*., (2007) found that textile industries are the largest consumers of organic dyes and estimated around 10 – 15 % of the dyes used are lost during the dyeing process and released into the effluents.

Synthetic dyes are an important class of chemicals which are used in many industrial processes. Synthetic dyestuffs have complex chemical structures which is not easy to degrade biologically. Evenmore, many industrial many biodegradability studies on dyes have showed that they are not likely biodegradable (Bali, 2004). Most of the dyestuffs are highly structured polymers with low biodegradability (Rauf *et al*., 2008). Dyes also usually contain elements such as nitrogen, chlorine, and sulphur. The oxidation products of these elements may be higher in toxicity than the parent molecule (Chen *et al.*, 2005). Particularly, synthetic dyes contribute to special environmental concern due to their degradation products such as aromatic amines which are considered as highly carcinogenic substances (Bali *et al*., 2004).

The wastewater containing dyes have a great variety of organic contaminants in a wide range of concentration especially in textile industry where they are highly coloured and complex variable of nature (Kurbus *et al*., 2003). The disposal of these coloured wastewaters poses huge problem for industry as well as a threat to the environment (Behnajady *et al*., 2006). It is because many of dyes are hard to be removed as they are stable to light and heat and are biologically non-degradable (Chen *et al*., 2005). In addition, the coloured dye effluents are considered to be highly toxic to aquatic life and and affect the symbiotic process by disturbing the natural equilibrium and reducing photosynthetic activity and primary production due to the colorization of water.

Effluents also contain significant level of organic contaminants which is toxic as they create odor, bad taste, unsightly colour and foaming (Ravikumar *et al*., 2006). Thus decoloration of effluents from dyes industrial application was happened to be a very important because of aesthetic and environmental concerns (Attia *et al*., 2008).

2.1.3 Rose Bengal (Acid Red 94)

Acid Red 94 (AR 94) is a tetraiodo-substituted dye of the xanthene class of dyes. It exhibits unusual spectroscopic and photochemical properties including a huge absorption coefficient in the visible region and a high tendency for intersystem crossing to produce a photochemically active triplet excited state. Figure 2.1 shows the molecular structure of Acid Red 94.

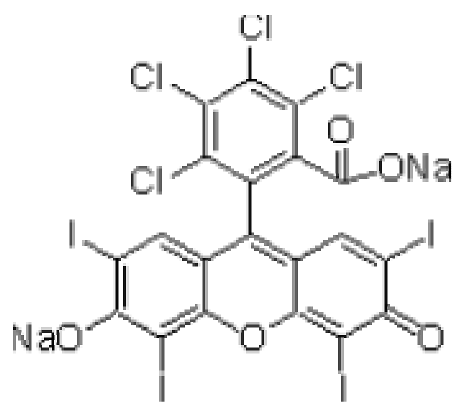

Figure 2.1: Molecular structure of Acid Red 94

The dye has been applied in photodynamic inactivation of catalase (Kim *et al.*, 2001), photoinactivation of $NADP^+$ via the production of singlet oxygen (Kim *et al.*, 2004), as a photosensitizing agent for inactivating biological species such as vaccinia virus, microsomal glucose-6-phosphatase (Lenard *et al.*, 1993), tripsin, *Escherichia coli* (Kita *et al.*, 1984), acetylcholinesterase, and HL-60 cells (Schäfer *et al.*, 2000).

2.2 Degradation of Dyes

2.2.1 Conventional Methods

Many efforts have been devoted to develop technologies that are able to minimize the hazardous effects caused by dye based industrial activities. The many different conventional methods applied in industrial wastewaters, such as coagulation and flocculation, membrane separation (ultra filtration, reverse osmosis) or elimination by activated carbon adsorption are not sufficient enough. This is because these process likely to produce a secondary pollutant or dumped as such (Körbahti and Rauf, 2008b). Then, this secondary pollutant is either sorbed or trapped in bioflocs. Thus, ecosystems of streams can be seriously affected (Bali, 2004). The United States Environmental Protection Agency's (U.S. EPA) Water Engineering Research Laboratory first reported that 11 out of 18 studied azo dyes (synthetic dyes) were substantially unaffected by the activated sludge process. Generally, adsorption onto activated carbon or chemical coagulation was applied to deal with wastewater containing dyes. However, these treatments also mainly transferred the contaminant from wastewater into solid wastes that the spent activated carbon and coagulant sludge need further and ultimate disposal eventually (Shu *et al*., 2004).

Removal of dyes from effluents in an economic way remains a huge problem for textile industries. Adsorption technique is an excellent way to treat effluents more than other conventional process, especially from the environmental point of view (Ravikumar *et al*., 2006). Carbon is being used as an adsorbent because of its high efficiency in treating the organic materials in effluents. Although it possessed high efficiency, but the enhancement of the price of activated carbon result in increasing the cost for its operation (Khattri and Singh, 1998). Biological treatment of wastewater can eliminate the important organic compounds. However, the biochemical decomposition by conventional method does not adequate enough to completely decolorize dye effluents

(Çatalkaya and Şengül, 2006). Thus it is a necessity for dyes based industry to reconsider upon the alternative method in their wastewater treatment.

2.2.2 Advanced Oxidation Processes

Advanced oxidation processes (AOPs) are alternative methods for decolorizing and reducing wastewater effluents generated by industries (Abdullah *et al*., 2008). AOPs are effective for detoxification and mineralization of the effluents from textile dyeing mills (Szpyrkowicz *et al*., 2001). Behnajady *et al*., (2006) stated that AOPs also a non-destructive physical water treatment processes, because they are able to eliminate compound rather than changing them into another medium such as solid waste. The use of AOPs, like UV/H_2O_2 (Körbahti and Rauf, 2008a), photocatalytic (Attia *et al*., 2008), Fenton and photo-Fenton processes (Çatalkaya and Şengül, 2006), has shown promising results as these processes appear to have the ability to completely decolorize and partially mineralize the textile industry dyes in short reaction time (Rauf *et al*., 2008), (Körbahti and Rauf, 2008b) and (Bali *et al*., 2004).

Among the AOPs, chemical oxidation using UV in the presence of H_2O_2 is a very promising technique. Process involving the use of UV radiation and H_2O_2 are characterized by the generation of hydroxyl radicals (Behnajady *et al*., 2006). UV wavelengths of 200 – 280 nm lead to dissociation of H_2O_2, with mercury lamps emitting at 254 nm being the most used. UV/H_2O_2 systems generate hydroxyl radicals (●OH) which are highly powerful oxidizing agents. Hydroxyl radicals can oxidized organic compounds (RH) producing organic radicals (●R), which also highly reactive and can be further oxidized (Bali *et al*., 2004). These radicals can then attack the dye molecules to undergo a series of reactions in which the organic molecules will be eliminated or converted into a simple molecules or harmless compound (Abdullah *et al*., 2007). The main reaction that occurs during UV/H_2O_2 oxidation process is as follows:

$$H_2O_2 + UV \rightarrow 2OH\bullet \qquad (2.1)$$

$$H_2O_2 \leftrightarrow HO_2^- + H^+ \qquad (2.2)$$

$$RH + OH\bullet \rightarrow H_2O + R\bullet \rightarrow \text{further oxidation} \qquad (2.3)$$

where R is the carbon chain.

The hydroxyl radicals will oxidize organic compounds producing organic radicals, which also a highly reactive and can undergo further oxidation. When generated, these radicals will react quickly and usually randomly with most organic compounds. The resulting organic radicals then reacts with oxygen to initiate series of degradative oxidation reaction that lead to mineralization of products such as CO_2 and H_2O (Çatalkaya and Şengül, 2006). The other possible reactions that may occur during the UV/H_2O_2 process are hydrogen abstraction, electrophilic addition and electron transfer reactions (Behnajady *et al*., 2006). Although AOPs have much kind of advantages in dyes decoloration, one major problem in AOPs is the high energy demand for UV lamps which lead to high operational cost. In order to minimize the irradiation time, energy consumption, and operational cost, there is necessary to optimize the pH condition, chemical types, chemical concentration, and pollutant/oxidant ratio, therefore are very important (Çatalkaya and Şengül, 2006). For this aim, the application of experimental design is the best solution where it will be used to optimize the important parameters that affected the efficiency of dyes decoloration.

2.3 Experimental Design Application

2.3.1 Introduction

Experimental design is a very important application in chemometrics (application of mathematical or statistical methods to chemical data), because chemical experiments have to be performed to get more knowledge about a process or system. The science is dependent on the experiments while the experimental design is to improve the experimental works. Experimental design methodology is used to decide which experiment needs to take place in order to get information on the certain chemical processes or products. It is used to determine which factors have an influence on the process output. Another application is to decide on how many experiment need to take place in order to obtain adequate knowledge on the desired product and the system. Thus, it is clear that optimization is an essential in chemical or biochemical processes (Kurbus *et al*., 2003).

In the other hand, the main objective of the experimental design (DoE) is to determine, with a minimum effort (less number of experiments), the effects of the different factors and their interactions in the process response, within the range of the studied variables. Besides, the information obtained allows deciding which factors and/or interactions are statistically significant. For that purpose, statistical techniques need to be used such as analysis of variance (ANOVA).

2.3.2 Screening

According to Kurbus *et al*., (2003), main methods of experimental design are factorial design including full factorial, fractional factorial design, orthogonal design (OD), D-optimal design, and uniform design. The selection of experiments has particular influence on the system. It is applied to determine the conditions to get the product of a process with desirable characteristics. The characteristics of the product named as a response. The factors that affecting the product are called independent variables while the product or the response is called dependent variables. So, experimental design is a set of carefully planned experiments.

The main step in experimental design is to choose the initial factors and response and to select the experimental domain. After screening process, unimportant factors are discarded and type of experimental design is determined beforehand. Normally, two level factorial designs are used for determination of significance parameters and intervals. The optimal response usually the lowest or the highest value of process output or response. After determining which factors have minimal or insignificance on the response, the optimum settings of the significant parameters levels that produce the best response need to be performed.

2.3.3 Optimization

According to Çatalkaya and Şengül, (2006), in order to find the optimum reaction conditions and to study the effect of significance parameters of dyes decoloration, the response surface methodology (RSM) was used. RSM essentially is the set of mathematical and statistical methods for designing experiments, building models, evaluating the effect of variables, and finding the optimum conditions of variables to predict the target responses (Myers and Montgomery, 2002). It is an important branch of experimental design and a critical tool in developing new processes, optimizing their performance, and improving design and formulations of new products (Körbahti and Rauf, 2008a). RSM is used for the analysis of dependent variables as functions of independent variables. Response surface procedures are not only primarily used for the purpose of allowing the researchers in order to understand the mechanism of the system or process but the most importantly is to determine the optimum operating conditions or to determine a region for the factors at a certain operating specification (Rauf *et al*., 2008).